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Naumann, David N; Beaven, Alastair; Dretzke, Janine; Hutchings, Sam; Midwinter, Mark J

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Searching for the optimal fluid to restore microcirculatory flow dynamics after haemorrhagic shock: a systematic review of preclinical studies

Running head: Fluids to restore the microcirculation

Authors and affiliations:

1. David N Naumann

National Institute of Health Research, Surgical Reconstruction and Microbiology
Research Centre, Queen Elizabeth Hospital, Birmingham, UK

Email: david.naumann@nhs.net

2. Alastair Beaven

National Institute of Health Research, Surgical Reconstruction and Microbiology
Research Centre, Queen Elizabeth Hospital, Birmingham, UK

Email: Alastair.Beaven@uhb.nhs.uk

3. Janine Dretzke

National Institute of Health Research, Surgical Reconstruction and Microbiology
Research Centre, Queen Elizabeth Hospital, Birmingham, UK.

School of Health & Population Sciences, College of Medical & Dental Sciences,
University of Birmingham, Birmingham, UK

Email: J.Dretzke@bham.ac.uk

4. Sam Hutchings

Kings College Hospital NHS Foundation Trust, London, UK

Email: sam.hutchings@kcl.ac.uk

5. Mark J Midwinter

National Institute of Health Research, Surgical Reconstruction and Microbiology

Research Centre, Queen Elizabeth Hospital, Birmingham, UK

Email: m.midwinter@bham.ac.uk

Corresponding Author

Mr David N Naumann, NIHR Surgical Reconstruction and Microbiology Research
Centre, Queen Elizabeth Hospital, Birmingham, B15 2TH, United Kingdom.

Email: david.naumann@nhs.net

Tel: +44 (0) 7861 242 807

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ABSTRACT

Background

Increased microcirculatory flow and perfusion has been reported to improve clinical outcomes following shock. The optimal resuscitation fluid to restore the flow dynamics of the microcirculation is unknown. This review summarises the preclinical literature in order to inform the direction and most important hypotheses for future clinical interventional studies.

Methods

Standard systematic review methodology was utilized, and registered with the Collaborative Approach to Meta Analysis and Review of Animal Data from Experimental Studies (CAMARADES). Medline and Embase (via OVID SP) and SCOPUS were searched for all preclinical studies of haemorrhagic shock that compared fluid resuscitation of any kind (e.g. blood products, crystalloids, colloids, or haemoglobin based oxygen carriers) to another fluid or haemorrhage only, and reported at least one microcirculatory physical endpoint (such as flow rate, velocity, vessel diameter, functional capillary density or glycocalyx thickness). Risk of bias was assessed using the Systematic Review Centre for Laboratory animal Experimentation (SYRCLE) tool. Translatability was also assessed for each study based on the most common recommendations.

Results

There were 3103 potential studies of interest, of which 71 studies fulfilled all eligibility criteria. There were 62 rodent, 5 canine and 4 porcine studies. Flow rate, velocity, and vessel diameter were the most commonly reported endpoints. Studies reported

the importance of the presence of haemoglobin, as well as osmotic potential and viscosity in providing optimal restoration of microcirculatory flow dynamics. Others reported the restoration of the endothelial glycocalyx and attenuation of inflammation as important properties for the choice of fluid. All studies were at potential risk of bias due to unclear randomization, concealment, and blinding. There were important threats to translatability for all studies.

Conclusion

The ideal resuscitation fluid for restoration of the microcirculation following haemorrhagic shock is likely to contain a preparation of haemoglobin, favour higher oncotic potential and viscosity, protect and reconstitute the endothelium, and attenuate inflammation. These hypotheses that are derived from preclinical research warrant further exploration in the clinical context.

Keywords: Microcirculation; haemorrhagic shock; resuscitation; preclinical

Systematic review registration

This protocol for this review has been registered with the Collaborative Approach to Meta Analysis and Review of Animal Data from Experimental Studies (CAMARADES) (<http://www.dcn.ed.ac.uk/camarades/research.html#protocols>), and published Open Access (Naumann DN, Dretzke J, Hutchings S, Midwinter MJ. Protocol for a systematic review of the impact of resuscitation fluids on the microcirculation after haemorrhagic shock in animal models. *Syst Rev.* 2015;4:135).

INTRODUCTION

Alterations in the microcirculation have been reported as more reliable than global parameters in predicting clinical outcome following septic shock(1) and traumatic haemorrhage(2). Improved microcirculatory flow during resuscitation is associated with reduced organ failure even when there is no difference in global haemodynamic factors(3). Even when global parameters are improved following shock, this may not be associated with improvements in the microcirculation and tissue perfusion(4). In such circumstances there appears to be a clinically meaningful discrepancy between the macro and microcirculatory behaviour. During normal physiological conditions both microcirculatory flow and tissue perfusion are determined by the circulatory pressure and volume. This phenomenon is known as 'haemodynamic coherence'(5). This coherence may be lost during circulatory shock, and therefore global surrogate markers may no longer be relied on as markers of microcirculatory function in that context – a rationale for monitoring of the microcirculation following shock.

During resuscitation of patients with haemorrhagic shock, fluid delivery is intended to increase oxygen delivery to tissues to meet demand, repay oxygen debt, eliminate lactate, and normalise pH. These processes all occur at the level of the microcirculation, representing a key anatomical location during shock and resuscitation. Although preclinical studies have been conducted to measure global haemodynamic parameters (such as blood pressure and heart rate) following haemorrhagic shock and resuscitation(6, 7), these surrogate markers of microcirculatory flow may not be relevant in the case of loss of haemodynamic coherence. Microcirculatory flow and dynamics therefore represent relevant study

endpoints for the assessment of resuscitation fluid delivery after haemorrhagic shock.

Current clinical resuscitative practice favours the utilisation of blood products (rather than crystalloid fluids) following haemorrhagic shock, but there are no randomised clinical studies that compare the microcirculation during different fluid resuscitation regimes. In order to guide clinical investigation and form credible hypotheses for testing in clinical research, it is timely to review the preclinical literature and determine the current state of evidence. No previous systematic reviews on this topic were identified during preliminary searches on MEDLINE and the Cochrane library. We hypothesised that provision of haemoglobin and plasma, in particular by whole blood, may be superior to other fluid characteristics in the restoration of the microcirculation following haemorrhagic shock.

Aim

The current systematic review aims to examine all available preclinical studies of haemorrhagic shock that use microcirculatory parameters as research endpoints and compare the efficacy of at least one type of fluid for resuscitation.

METHODS

This systematic review is intended to address the impact of resuscitation fluids on the behaviour of the microcirculation in animal models of haemorrhagic shock.

The protocol for this systematic review has been published previously(8), and made freely available through Open Access and registration at the Collaborative Approach to Meta Analysis and Review of Animal Data from Experimental Studies (CAMARADES)(9). Systematic review methodology is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines(10).

Study subjects

This systematic review includes studies that utilise animal models of haemorrhagic shock (any size, age, strain and species). Any volume of haemorrhage (survivable or non survivable) was allowed as long as the intention was to create a period of circulatory shock after which fluid resuscitation was delivered. Study protocols with additional elements such as trauma were still eligible for inclusion. Studies that utilised isovolaemic exchange transfusion, ischaemia-reperfusion, or septic shock models were excluded unless they also contained a subgroup of haemorrhagic shock.

Interventions

Studies that used at least one type of fluid intervention for resuscitation following haemorrhage were eligible for inclusion. There were multiple interventions of interest, broadly categorised as: (i) blood products (e.g. whole blood, packed red cells (PRBCs), plasma); (ii) haemoglobin-based oxygen carriers (HBOC) (e.g.

modified bovine haemoglobin); (iii) crystalloids (e.g. Ringer's lactate, 0.9% or hypertonic saline); and (iv) colloids (e.g. albumin, dextran, starch).

Comparisons

The studies included in this systematic review were varied in both methodology and research question. Multiple permutations of fluid comparisons were made (for example blood product versus crystalloid, and colloid versus crystalloid). Some studies use haemorrhagic shock alone as a control, and some use surgical instrumentation (sham) as a control. These comparisons are summarised in narrative form.

Outcomes

The outcomes of interest included any parameter that was intended to represent the physical microcirculatory behaviour. These included: flow rate (nL/s); red blood cell velocity (mm/s), vessel diameter (μm); Functional capillary density (%); glycocalyx thickness (μm); Shear rate (s^{-1}); proportion of perfused vessels (%); vessel density (n/mm); perfused vessel density (n/mm); microcirculatory flow index; blood flow intensity; and heterogeneity index. Studies that only examined physiological aspects of the microcirculation such as lactate, oxygen partial pressures, and delivery of oxygen but did not report physical (flow dynamics) parameters(11-19) were excluded.

Study design

Studies were included if they were controlled prospective animal studies with detailed reporting of the type and amount of fluid(s) used and at least one

microcirculatory physical parameter. Although randomised studies with blinded outcome assessment were considered preferable, prospective studies without such design were still eligible for inclusion. Single case reports and letters were rejected. Conference proceedings and abstracts were screened for new data and adequate methodological detail. They were only included if they contained new data (not replicated by full papers from the same authors and time period). Uncontrolled studies were recorded but not included in the analysis.

Search strategy

The detailed search strategy for this systematic review has been published previously(8). In short, Embase, Medline and SCOPUS were searched to identify eligible studies. A combination of terms were used that referred to the model (e.g. “haemorrhage”, “shock”), the intervention (e.g. “transfusion”, “fluid”, “resuscitation”), and the endpoint (e.g. “microcirculation”, “endothelium”). An example search is included in the prior publication(8). There were no restrictions applied to study type, date, or language. Reference lists of included studies and relevant reviews were screened for further eligible studies.

Study Selection

All titles and abstracts were screened by two independent reviewers, and full texts were obtained for studies that appeared to be of interest. Eligible studies were identified from reading the full texts.

Data extraction

Data extraction was performed by one reviewer (D.N.N.) and confirmed by another (A.B.). Data were extracted with regards to study characteristics and design (author, year, type of study, hypothesis), animal model (species, age, experimental groups, size/weight, housing), number of animals (haemorrhagic shock and resuscitation only), and haemorrhage protocol (technique, percentage and volume of bleeding, timings, and target pressures). Details regarding interventions (type and timings), and microcirculatory monitoring (technique, anatomical location) were also extracted. A summary of the extracted data fields is shown in Supplementary Table 1.

Quality assessment

Two reviewers (D.N.N. and A.B.) assessed the included studies based on the Systematic Review Centre for Laboratory animal Experimentation (SYRCLE) risk of bias tool(20). This tool assesses selection, performance, detection, attrition, and reporting biases.

Translatability

The validity of study design with regards to translatability to clinical practice was examined by two authors (D.N.N. and A.B.) for each study based on the three domains described by Henderson et al: (i) threats to internal validity; (ii) threats to construct validity; and (iii) threats to external validity(21). The domain relating to outcome measure validity was omitted since this systematic review only includes studies with pre-defined outcome measures (microcirculatory physical parameters) that are considered valid endpoints of shock resuscitation.

RESULTS

Search results

Figure 1 shows the PRISMA diagram for the study selection. The initial search strategy identified 3103 studies, from which 369 full texts were examined. There were 71 studies that measured the impact of resuscitation fluids on the microcirculation in an animal model of haemorrhagic shock(22-92).

Study characteristics

The 71 included studies were published between 1990 and 2015, and include 67 original articles and 4 conference proceedings/abstracts with 45 individual first authors. Countries of origin are listed as: Austria, Brazil, Canada, China, France, Germany, Hungary, Italy, Japan, Spain, Taiwan, and the USA. All studies were prospective, experimental controlled studies. Although random allocation of intervention and control arms was *implied* by all studies, the words “random” or “randomly” were only explicitly reported in 52/71 (73%) of studies. The extracted

Animal characteristics

All studies included a single species experimental model. Animal characteristics are summarised in Table 1, and included 62 rodent, 5 canine and 4 porcine studies. A total of 1959 animals underwent haemorrhagic shock and resuscitation in the included studies. There were 55 studies that reported the sex of the animals, of which 48 included only male animals, 4 studies had mixed male and female animals, and 3 included only female animals.

Haemorrhagic shock protocols

A haemorrhagic shock protocol was described in all studies, and was heterogenous, as illustrated in Table 2. Some studies bled the animals to target values or percentage of mean arterial pressures, whereas others bled to a percentage of total blood volume or weight. The length of time of the “shock” phase was also variable.

Microcirculatory monitoring techniques

Several different techniques and anatomical locations were used to determine microcirculatory parameters, as summarised in Table 3. Five studies(41, 56, 69, 88, 89) examined the microcirculation in multiple regions, and the remainder studied only one anatomical location. One study conducted haemorheological analysis externally (from blood samples) without specific anatomical location(92). Some studies used a combination of techniques at the same anatomical location(40, 58). Intravital microscopy was the most common technique used for microcirculatory visualisation, with dorsal skin fold and bowel/mesentery being the most common anatomical locations. Only five studies used sidestream darkfield microscopy (SDF)(43, 44, 58, 61, 69), which is considered the most appropriate technique for clinical assessment(93).

Fluid comparisons

There were multiple comparisons made between fluids in the included studies, summarised in Supplementary Table 2. There were 29 permutations of sham (no haemorrhage), haemorrhage only, blood product, HBOC, crystalloid and colloid fluid administration.

Risk of bias

The majority of studies fulfilled SYRCLE criteria to control for risk of bias by specifically reporting a random allocation sequence generation for the different interventions, and also reporting similar baseline characteristics (illustrated in Supplementary Table 3). No studies met all SYRCLE criteria. All studies were at risk of selection bias due to lack of reporting of allocation concealment, and performance bias due to lack of reporting of blinding of caregivers/investigators. All studies were also at risk of detection bias due to lack of reporting of random outcome assessment.

Translatability

Assessment of the translatability of the animal model to clinical relevance found some consistently under-reported details, which threaten the validity of the studies in humans (illustrated in Supplementary Table 4). Most of the studies did not describe a power calculation in their methodology, did not have blinded outcome assessment, and did not include a dose-response relationship. Only one study reported a rationale for the age of the animals(43), and none reported their experimental model had been tested with different transgenic strains, different species, or in collaboration with different research groups (for the same experiment).

Data synthesis

An assessment of feasibility of meta-analysis was made, but was considered to be unwarranted due to high heterogeneity of haemorrhage protocol (5 permutations), interventions (29 permutations), and endpoints (6 permutations) between studies. In particular there were variations in study hypotheses and research questions that rendered meta-analytic synthesis impossible. Descriptive narrative is therefore

utilised to synthesise findings across similar studies. Studies were summarised according to study hypothesis and research question. The included studies could be broadly divided into 5 hypotheses as summarised in Tables 4–8. These primarily investigated fluids containing haemoglobin, physical properties (viscosity and oncotic/osmotic potential), and the restorative and anti-inflammatory properties of the resuscitation fluids. Some studies reported data related to more than one of these hypotheses. Of these studies, 55/71 (77.5%) reported one fluid being superior to another, with the remainder reporting equivalence between fluids or only testing one fluid.

a. Fluids containing haemoglobin

There were 27 studies that considered resuscitation fluids containing haemoglobin for the restoration of microcirculatory flow dynamics as summarised in Table 4.

There were 21 studies that examined HBOC fluids, including 10 studies that tested HBOC versus whole blood and non haemoglobin (Hb) carrying fluids; 8 studies that tested HBOC versus non Hb carrying fluids; and 3 studies that tested HBOC versus whole blood only. HBOC preparations included bovine haemoglobin(24, 25, 34, 78), modified human haemoglobin(87), mixed human/bovine haemoglobin(65), diaspirin cross-linked haemoglobin (DCLHb) (46, 56, 62, 83), o-raffinose cross-linked oligomerized haemoglobin(52), and nitric oxide-scavenging recombinant haemoglobin(47). In the 13 studies in which HBOC fluids were directly compared to whole blood, three HBOCs (bovine haemoglobin glutamer-250(63), modified human haemoglobin(87), and DCLHb(62)) were superior to whole blood in the restoration of microcirculatory flow dynamics. All except one(71) of the remaining studies reported that HBOC fluids were equivalent to whole blood in terms of flow dynamics. HBOC

fluids were superior to non Hb carrying fluids in 13 studies, and equivalent to non Hb carrying fluids in 5 studies.

Some of the studies that tested HBOC fluids also addressed the potential unwanted side effects of HBOCs such as vasoconstriction, nitric oxide (NO) scavenging and leucocyte/endothelial interactions. They reported that HBOCs did not cause vasoconstriction(25), did not increase leucocyte/endothelial interactions(25, 49, 62, 83), and do not have toxic or lethal effects(38) when compared to other non-HBOC fluids. Some preparations of Hb are superior to others(65), and lower Hb concentrations appeared to be superior to higher concentrations with regards to vasoconstriction(34, 63). Furthermore specific modification of HBOCs to reduce NO scavenging has been reported as effective(47). Conversely, one study did report hepatotoxic effects of HBOC(55).

There were 6 studies that tested fluids containing Hb that were not HBOCs. These included 4 studies testing whole blood versus crystalloid(35, 51, 61) or colloid(70), and two studies examining preparations of PRBCs(68, 79). These studies reported that whole blood was superior to crystalloid and colloid. When PRBCs were tested, one oxygen carrying emulsion (perflubron emulsion) was reported as being superior to red cells(68). Another study reported that lower oxygen affinity PRBCs were superior to higher oxygen affinity(79).

b. Osmotic and oncotic potential

There were 19 studies that tested the hypothesis that the osmotic/oncotic properties of a resuscitative fluid are most important in the restoration of the microcirculation following haemorrhage, of which 14 studies reported findings in keeping with this hypothesis. These are summarised in Table 5. Hypertonic-hyperosmotic solutions of

saline-dextran(59), saline-HES(64, 77, 81), and HES(42, 82) were reported as superior to isotonic solutions. One study reported that hypertonic saline/dextran fluid improved microcirculatory parameters better than whole blood(72). One study reported that hypertonic saline resuscitation was superior to isotonic fluid but only if whole blood was also returned to the animal(91). Increase colloid pressure and volume expansion was reported as superior for microcirculatory restoration when comparing colloid to crystalloid solutions(53, 88), and when using modified colloids(29). One study reported that the duration of time of oncotic force was important in restoring the microcirculation(28), and another showed that hypertonic solutions may restore microcirculatory flow for longer than isotonic solutions(41). One study reported that hypertonic fluid is superior due to its reduced effects on red blood cell deformability when compared to isotonic fluids(92).

Five studies did not report superiority of higher osmotic/oncotic potential fluids; these included reports of equivalence(23) or inferiority(50). Although some studies reported that the microcirculatory fluid dynamics were unaffected by higher oncotic/osmotic properties when compared to isotonic fluids, it was noted that the permeability of microvessels(66, 67) and haemoglobin oxygen saturation(58) may be improved with such solutions nevertheless.

Some studies reported that the hypertonic-hyperosmotic nature of the resuscitative fluids influenced the behaviour of leucocytes to a greater effect than isotonic solutions in their actions towards improving the microcirculation(66, 67, 81), as described later.

c. Viscosity

There were 12 studies which tested the hypothesis that increased fluid viscosity was superior to normal or reduced viscosity in the restoration of microcirculatory flow following haemorrhage, as summarised in Table 6. Ten of these studies had findings in keeping with this hypothesis. Higher viscosity preparations of hydroxylethyl starch (HES) are reported as superior to lower viscosity HES(26, 33, 85). Higher viscosity preparations of Ringers Lactate (by addition of 0.3% alginate) were superior to conventional Ringers Lactate in restoring the microcirculation(80). Solutions with increased molecular weight (with higher viscosity) were show to be superior to lower molecular weight (and therefore lower viscosity) solutions; for example using higher density polymerised human serum albumin (HSA)(60) or higher molecular weight HES(32).

The viscosity—rather than the oxygen carrying capacity—has been reported as the factor of importance even when using oxygen-carrying solutions(27, 31). Furthermore increase in viscosity was reported as more important than the increase in oncotic pressure(30). High viscosity preparations of pegylated bovine albumin were superior than the same preparations combined with red blood cells, demonstrating that transfusion haemoglobin triggers might be lowered if higher viscosity fluids are used(86).

Two studies did not find that higher viscosity was superior to lower; one of these compared higher and lower viscosity HBOCs(69) and another higher versus lower viscosity non-oxygen carrying fluids(45).

d. Attenuation of inflammation

There were 9 studies that considered the anti-inflammatory properties of resuscitation fluids, as summarised in Table 7. Using albumin as a resuscitative fluid has been reported to both improve the microcirculatory parameters as well as reducing the inflammatory response(48). A number of studies have proposed that small volume resuscitation with hypertonic-hyperosmotic solutions may affect the flow behaviour of leucocytes and reduce their stagnation(40), margination(22), rolling(67) and adhesion to the endothelium(81), as well as attenuating the number of endothelium-leukocyte interactions(23, 66, 90). Reduction of leucocyte adhesion has also been reported when using gelatine serum protein solutions as a resuscitative fluid(57).

e. Restorative properties

There were 5 recent studies from the USA that tested the hypothesis that endothelial glycocalyx is shed following haemorrhagic shock, and may be restored by components of plasma (but not crystalloid), restoring the microcirculatory dysfunction. All studies reported that their results were in keeping with that hypothesis as summarised in Table 8 (54, 73-76).

Notable exclusions

Some studies were ineligible for inclusion due to lack of comparator. This was either due to the same fluid being given in different volumes(94) or no control(95). Some study protocols varied the amount of haemorrhage rather than resuscitation fluid(96, 97). Isovolaemic exchange transfusion(98-100), haemodilution(101), and ischaemia-

reperfusion(102-105) protocols were excluded. Small volume acute blood loss without haemorrhagic shock(106, 107) were also excluded.

There were multiple studies that used drug delivery as interventions (rather than purely comparing different fluids), including additives(108-111), noradrenaline(112), polydatin(113), nitric oxide(114) and naloxone(115).

Although of interest in the basic science of endothelial behaviour, in vitro studies(116) and those that measured endothelial relaxation(117, 118) and activity(119) were excluded. Similarly, conformational changes in red blood cells (such as deformability and fragility(99, 106)), and the modulation of the inflammatory components of haemorrhagic shock(120) and leucocyte behaviour(48, 81) were ineligible for inclusion.

Studies that only reported perfusion endpoints (such as delivery of oxygen) rather than any microcirculatory flow dynamics were also excluded(17, 18).

DISCUSSION

According to the preclinical available evidence, the most favourable properties of resuscitative fluids for the restoration of the microcirculation are: (i) the presence of a haemoglobin preparation (HBOC being mostly equivalent to whole blood); (ii) higher viscosity; (iii) higher oncotic/osmotic potential, and (iv) having the physical and constituent properties that enable attenuation of endothelial-leucocyte interactions, reduced inflammation and endothelial permeability. The evidence for these properties comes from 71 published preclinical studies that have each tested the basic scientific questions regarding physical properties of resuscitation fluids, as well as the influence of their constituents. Since none have tested all of these properties

in a single experiment, it is only by summation and consideration of all available evidence that translatable research questions might be considered for the clinical context.

After catastrophic haemorrhage whole blood is not usually readily available, and fractionated parts of blood such as PRBCs, FFP, and platelets are precious resources. Furthermore the most appropriate ratios of these fractions is a matter of controversy(121). Availability is also not the only limitation, since whole blood or components may not be the ideal fluids to deliver following haemorrhagic shock; some of these preclinical studies have demonstrated superiority of other fluid strategies to delivery of whole blood. Regardless of the type of fluid delivered in the emergency scenario, the priority is to restore tissue perfusion by enabling the transport of oxygen at the microcirculatory surface. This goal requires consideration of which characteristics of the resuscitative fluid are most important for that task. Not only should the fluid restore the microcirculatory flow dynamics, but may also contribute to the mitigation and repair of endothelial injury that has occurred following haemorrhage. Restoration of the endothelium and endothelial glycocalyx and prevention of leucocyte-endothelial interactions may be key for longer-term outcomes, but such a question has not been answered in animal models. All of the individual fluid characteristics reported here provide a sound basis for further clinical research.

The design of an 'ideal' fluid for resuscitation after haemorrhagic shock appears to depend on several factors of importance. The careful balance of osmotic potential and viscosity in resuscitative fluids appears to allow the fluid to inhibit endothelial cell

swelling, minimise shear stress, and keep the individual microcirculatory segments open long enough to allow the exchange of oxygen between the circulation and the end tissues. Some studies in this review have reported that Hb preparations and red cells improve the microcirculatory function by their osmotic and viscous effect on microcirculatory flow dynamics rather than their oxygen carrying capacity. Reduction in cell swelling and maladaptive endothelial-leucocyte interactions might lead to reduction in shunting of flow and subsequent systemic inflammatory response. The fluid itself has potential to deposit glycoproteins and essential components of the endothelial glycocalyx that may enable the microcirculation to improve its function. Although HBOCs are thought to interfere with the endothelium derived relaxing factor and NO system and increase vasoconstriction, unwanted oxidative reactions, and endothelial-leucocyte interactions, the studies included in this review appear to report that appropriate modification of HBOCs can reduce such unwanted effects.

As well as the usual limitations in translatability that arise when attempting to apply results of animal studies to the clinical context, the studies included in this review were all at risk of threats to their validity according to the most widely common recommendations for animal studies(21). The majority of studies were undertaken with rodent models rather than large animals, which may provide further issues for translatability; testing the same research question from a rodent study in a large animal study may result in a different answer(122). Furthermore all studies had a potential risk of bias according to the SYRCLE tool; indeed the majority of studies were only assessed as positive in 3 or fewer domains (out of a possible 10). In particular the general lack of blinding of investigators and outcome assessments mandate a cautious approach to their interpretation. It is not known whether these

studies failed to fulfil all domains of the assessment tools due to methodological deficiencies or whether they simply did not report the relevant details. If further animal studies are to be conducted in this field we would recommend that the experimental protocols *and reporting technique* are designed according to the SYRCLE tool domains of importance. This tool is based on the Cochrane Collaboration's Risk tool for assessing bias in randomised controlled trials, and should therefore be the gold standard for animal studies that hope to establish clinically sound hypotheses(123). It is also important to note that a limited number of studies used sublingual video-microscopy, which is the most appropriate technique for human translatability.

Limitations

Meta-analytical tests could not be undertaken for the studies included in this review since there were too many permutations of animal model, intervention, and outcome measure to provide consistent grouping of studies. Such a feasibility assessment was pre-defined in the original review protocol. Statistical heterogeneity could not be assessed and funnel plots could not be used to assess publication bias. This is a notable limitation since significant results are more likely to be published(124), and it is likely that unpublished and unavailable studies have not been included in the current systematic review. Nevertheless, this systematic review summarises the available published literature with regards to haemorrhagic shock resuscitation in preclinical models, and provides a basis on which to test hypotheses in the clinical context.

There are some clinically relevant omissions in the preclinical literature with regards to haemorrhagic shock resuscitation and the microcirculation. For example

there were no studies that tested platelets as a resuscitation fluid. When plasma was delivered and shown to be superior, the exact constituent components of benefit have not been identified. There were also no clinically relevant long-term outcomes analysed. The experimental protocols were not intended to assess clinically relevant outcomes such as 24-hour mortality or complications of treatment. Such questions could only be reliably tested in the clinical context.

Based on the available preclinical evidence, the ideal resuscitation fluid for restoration of the microcirculation following haemorrhagic shock is likely to contain a preparation of haemoglobin, favour higher oncotic/osmotic potential and viscosity, protect and reconstitute the endothelium, and attenuate inflammation. These hypotheses are derived from an extensive series of preclinical studies that have tested the basic biological questions regarding the physical properties of a wide range of fluids. Because of the potential risk of translatability, further evaluation in clinical studies are warranted in order to determine the ‘ideal’ resuscitative fluid to restore the microcirculation in humans.

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Figure Legends

Figure 1. PRISMA diagram to illustrate search results

References

1. De Backer D, Donadello K, Sakr Y, Ospina-Tascon G, Salgado D, Scolletta S and Vincent JL: Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome. *Crit Care Med* 41(3):791-9, 2013.
2. Tachon G, Harrois A, Tanaka S, Kato H, Huet O, Pottecher J, Vicaut E and Duranteau J: Microcirculatory alterations in traumatic hemorrhagic shock. *Crit Care Med* 42(6):1433-41, 2014.
3. Trzeciak S, McCoy JV, Phillip Dellinger R, Arnold RC, Rizzuto M, Abate NL, Shapiro NI, Parrillo JE and Hollenberg SM: Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis. *Intensive Care Med* 34(12):2210-7, 2008.
4. Dubin A, Pozo MO, Casabella CA, Palizas F, Jr., Murias G, Moseinco MC, Kanoore Edul VS, Palizas F, Estenssoro E and Ince C: Increasing arterial blood pressure with norepinephrine does not improve microcirculatory blood flow: a prospective study. *Crit Care* 13(3):R92, 2009.
5. Ince C: Hemodynamic coherence and the rationale for monitoring the microcirculation. *Crit Care* 19 Suppl 3:S8, 2015.
6. Turranoglu S, Kaya S, Kararmaz A and Turhanoglu AD: Lung perfusion in hemorrhagic shock of rats: the effects of resuscitation with whole blood, saline or Hes 6%. *Tohoku Journal of Experimental Medicine* 195(4):245-51, 2001.
7. Behrman SW, Fabian TC, Kudsk KA and Proctor KG: Microcirculatory flow changes after initial resuscitation of hemorrhagic shock with 7.5% hypertonic saline/6% dextran 70. *Journal of Trauma* 31(5):589-600, 1991.

8. Naumann DN, Dretzke J, Hutchings S and Midwinter MJ: Protocol for a systematic review of the impact of resuscitation fluids on the microcirculation after haemorrhagic shock in animal models. *Syst Rev* 4:135, 2015.
9. CAMARADES: Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies. Vol. 2016. 2016.
10. Moher D, Liberati A, Tetzlaff J and Altman DG: Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg* 8(5):336-41, 2010.
11. Knotzer H, Pajk W, Maier S, Dunser MW, Ulmer H, Schwarz B, Salak N and Hasibeder WR: Comparison of lactated Ringer's, gelatine and blood resuscitation on intestinal oxygen supply and mucosal tissue oxygen tension in haemorrhagic shock. *British Journal of Anaesthesia* 97(4):509-16, 2006.
12. Legrand M, Mik EG, Balestra GM, Lutter R, Pirracchio R, Payen D and Ince C: Fluid resuscitation does not improve renal oxygenation during hemorrhagic shock in rats. *Anesthesiology* 112(1):119-27, 2010.
13. van Bommel J, de Korte D, Lind A, Siegemund M, Trouwborst A, Verhoeven AJ, Ince C and Henny CP: The effect of the transfusion of stored RBCs on intestinal microvascular oxygenation in the rat. *Transfusion* 41(12):1515-23, 2001.
14. Sinaasappel M, van Iterson M and Ince C: Microvascular oxygen pressure in the pig intestine during haemorrhagic shock and resuscitation. *Journal of Physiology* 514(Pt 1):245-53, 1999.
15. Balkamou X, Xanthos T, Stroumpoulis K, Moutzouris DA, Rokas G, Agrogiannis G, Demestihia T, Patsouris E and Papadimitriou L: Hydroxyethyl starch 6% (130/0.4) ameliorates acute lung injury in swine hemorrhagic shock. *Anesthesiology* 113(5):1092-1098, 2010.

16. Boura C, Caron A, Longrois D, Mertes PM, Labrude P and Menu P: Volume expansion with modified hemoglobin solution, colloids, or crystalloid after hemorrhagic shock in rabbits: effects in skeletal muscle oxygen pressure and use versus arterial blood velocity and resistance. *Shock (Augusta, Ga.)* 19(2):176-182, 2003.
17. Van Iterson M, Sinaasappel M, Burhop K, Trouwborst A and Ince C: Low-volume resuscitation with a hemoglobin-based oxygen carrier after hemorrhage improves gut microvascular oxygenation in swine. *Journal of Laboratory and Clinical Medicine* 132(5):421-431, 1998.
18. van Iterson M, Siegemund M, Burhop K and Ince C: Hemoglobin-based oxygen carrier provides heterogeneous microvascular oxygenation in heart and gut after hemorrhage in pigs. *J Trauma* 55(6):1111-24, 2003.
19. van Iterson M, Bezemer R, Heger M, Siegemund M and Ince C: Microcirculation follows macrocirculation in heart and gut in the acute phase of hemorrhagic shock and isovolemic autologous whole blood resuscitation in pigs. *Transfusion* 52(7):1552-9, 2012.
20. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M and Langendam MW: SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol* 14:43, 2014.
21. Henderson VC, Kimmelman J, Fergusson D, Grimshaw JM and Hackam DG: Threats to validity in the design and conduct of preclinical efficacy studies: a systematic review of guidelines for in vivo animal experiments. *PLoS Med* 10(7):e1001489, 2013.

22. Bauer M, Feucht K, Ziegenfuss T and Marzi I: Attenuation of shock-induced hepatic microcirculatory disturbances by the use of a starch-deferoxamine conjugate for resuscitation. *Critical Care Medicine* 23(2):316-22, 1995.
23. Bauer M, Marzi I, Ziegenfuss T, Seeck G, Buhren V and Larsen R: Comparative effects of crystalloid and small volume hypertonic hyperoncotic fluid resuscitation on hepatic microcirculation after hemorrhagic shock. *Circulatory Shock* 40(3):187-193, 1993.
24. Bi Z, He X, Zhang X, Jiang Y, Zhao K and Liu Q: Pharmacodynamic study of polyethylene glycol conjugated bovine hemoglobin (PEG-bHb) in rats. *Artificial Cells, Blood Substitutes, and Immobilization Biotechnology* 32(2):173-187, 2004.
25. Botzlar A, Nolte D and Messmer K: Effects of ultra-purified polymerized bovine hemoglobin on the microcirculation of striated skin muscle in the hamster. *European journal of medical research* 1(10):471-478, 1996.
26. Cabrales P, Intaglietta M and Tsai AG: Increase plasma viscosity sustains microcirculation after resuscitation from hemorrhagic shock and continuous bleeding. *Shock* 23(6):549-555, 2005.
27. Cabrales P, Intaglietta M and Tsai AG: Transfusion restores blood viscosity and reinstates microvascular conditions from hemorrhagic shock independent of oxygen carrying capacity. *Resuscitation* 75(1):124-134, 2007.
28. Cabrales P, Nacharaju P, Manjula BN, Tsai AG, Acharya SA and Intaglietta M: Early difference in tissue pH and microvascular hemodynamics in hemorrhagic shock resuscitation using polyethylene glycol-albumin- and hydroxyethyl starch-based plasma expanders. *Shock* 24(1):66-73, 2005.

29. Cabrales P, Tsai AG, Ananda K, Acharya SA and Intaglietta M: Volume resuscitation from hemorrhagic shock with albumin and hexaPEGylated human serum albumin. *Resuscitation* 79(1):139-146, 2008.
30. Cabrales P, Tsai AG and Intaglietta M: Hyperosmotic-hyperoncotic versus hyperosmotic-hyperviscous: small volume resuscitation in hemorrhagic shock. *Shock* 22(5):431-7, 2004.
31. Cabrales P, Tsai AG and Intaglietta M: Is resuscitation from hemorrhagic shock limited by blood oxygen-carrying capacity or blood viscosity? *Shock* 27(4):380-389, 2007.
32. Cabrales P, Tsai AG and Intaglietta M: Resuscitation from hemorrhagic shock with hydroxyethyl starch and coagulation changes. *Shock* 28(4):461-467, 2007.
33. Cabrales P, Tsai AG and Intaglietta M: Increased plasma viscosity prolongs microhemodynamic conditions during small volume resuscitation from hemorrhagic shock. *Resuscitation* 77(3):379-386, 2008.
34. Cabrales P, Tsai AG and Intaglietta M: Polymerized bovine hemoglobin can improve small-volume resuscitation from hemorrhagic shock in hamsters. *Shock* 31(3):300-307, 2009.
35. Casali R, Buti G, Cantini Q and Novelli GP: ["Small volume resuscitation" in hypovolemic rats. Effects on microcirculation]. *Minerva Anestesiologica* 68(1-2):17-24, 2002.
36. Cheung AT, Driessen B, Jahr JS, Duong PL, Ramanujam S, Chen PC and Gunther RA: Blood substitute resuscitation as a treatment modality for moderate hypovolemia. *Artif Cells Blood Substit Immobil Biotechnol* 32(2):189-207, 2004.
37. Cheung AT, Duong PL, Driessen B, Chen PC, Jahr JS and Gunther RA: Systemic function, oxygenation and microvascular correlation during treatment of

hemorrhagic shock with blood substitutes. *Clinical Hemorheology and Microcirculation* 34(1-2):325-334, 2006.

38. Cheung AT, Jahr JS, Driessen B, Duong PL, Chan MS, Lurie F, Golkaryeh MS, Kullar RK and Gunther RA: The effects of hemoglobin glutamer-200 (bovine) on the microcirculation in a canine hypovolemia model: a noninvasive computer-assisted intravital microscopy study. *Anesth Analg* 93(4):832-8, 2001.

39. Cheung AT, To PL, Chan DM, Ramanujam S, Barbosa MA, Chen PC, Driessen B, Jahr JS and Gunther RA: Comparison of treatment modalities for hemorrhagic shock. *Artificial Cells, Blood Substitutes, & Immobilization Biotechnology* 35(2):173-90, 2007.

40. Corso CO, Okamoto S, Ruttinger D and Messmer K: Hypertonic saline dextran attenuates leukocyte accumulation in the liver after hemorrhagic shock and resuscitation. *Journal of Trauma-Injury Infection & Critical Care* 46(3):417-23, 1999.

41. Cryer HM, Gosche J, Harbrecht J, Anigian G and Garrison N: The effect of hypertonic saline resuscitation on responses to severe hemorrhagic shock by the skeletal muscle, intestinal, and renal microcirculation systems: seeing is believing. *American Journal of Surgery* 190(2):305-313, 2005.

42. Gierer P, Vollmar B, Schaser KD, Andreas C, Gradl G and Mittlmeier T: Efficiency of small-volume resuscitation in restoration of disturbed skeletal muscle microcirculation after soft-tissue trauma and haemorrhagic shock. *Langenbecks Archives of Surgery* 389(1):40-5, 2004.

43. Gonzalez R, Urbano J, Lopez J, Solana MJ, Botran M, Garcia A, Fernandez SN and Lopez-Herce J: Microcirculatory alterations during haemorrhagic shock and after resuscitation in a paediatric animal model. *Injury* 47(2):335-41, 2016.

44. Gonzalez R, Urbano J, Solana MJ, Botran M, Garcia A, Lopez-Herce J and Lopez J: Evaluation of three resuscitation protocols in hypovolemic shock using microcirculation analysis in an animal model. *Archives of Disease in Childhood* 97:A298, 2012.
45. Guerci P, Tran N, Menu P, Losser MR, Meistelman C and Longrois D: Impact of fluid resuscitation with hypertonic-hydroxyethyl starch versus lactated ringer on hemorheology and microcirculation in hemorrhagic shock. *Clinical Hemorheology and Microcirculation* 56(4):301-317, 2014.
46. Gulati A and Sen AP: Dose-dependent effect of diaspirin cross-linked hemoglobin on regional blood circulation of severely hemorrhaged rats. *Shock (Augusta, Ga.)* 9(1):65-73, 1998.
47. Hermann J, Corso C and Messmer KF: Resuscitation with recombinant hemoglobin rHb2.0 in a rodent model of hemorrhagic shock. *Anesthesiology* 107(2):273-280, 2007.
48. Horstick G, Lauterbach M, Kempf T, Bhakdi S, Heimann A, Horstick M, Meyer J and Kempfski O: Early albumin infusion improves global and local hemodynamics and reduces inflammatory response in hemorrhagic shock. *Critical Care Medicine* 30(4):851-855, 2002.
49. Hungerer S, Nolte D, Botzlar A and Messmer K: Effects of diaspirin crosslinked hemoglobin (DCLHb) on microcirculation and local tissue pO₂ of striated skin muscle following resuscitation from hemorrhagic shock. *Artificial Cells, Blood Substitutes, and Biotechnology* 34(5):455-471, 2006.
50. Kao R, Jiao X, Xenocostas A, Rui T, Parry N and Martin C: Effects of normal saline (NS), Ringer's lactate (RL) and 7.5% hypertonic saline (HTS) with and without erythropoietin (EPO) on microcirculatory perfusion and tissue bioenergetics of the small

intestine in a hemorrhagic shock and resuscitation rat model. *Intensive Care Medicine* 37:S151, 2011.

51. Kao RL, Xenocostas A, Rui T, Huang W and Martin CM: The effect of erythropoietin on microcirculation perfusion and tissue bioenergetics of the small intestine in a hemorrhagic shock and resuscitation rat model. *Journal of Trauma-Injury Infection & Critical Care* 68(6):1342-8, 2010.

52. Kerger H, Tsai AG, Saltzman DJ, Winslow RM and Intaglietta M: Fluid resuscitation with O₂ vs. non-O₂ carriers after 2 h of hemorrhagic shock in conscious hamsters. *American Journal of Physiology - Heart and Circulatory Physiology* 272(1 41-1):H525-H537, 1997.

53. Komori M, Takada K, Tomizawa Y, Uezono S, Nishiyama K and Ozaki M: Effects of colloid resuscitation on peripheral microcirculation, hemodynamics, and colloidal osmotic pressure during acute severe hemorrhage in rabbits. *Shock* 23(4):377-82, 2005.

54. Kozar RA, Peng Z, Zhang R, Holcomb JB, Pati S, Park P, Ko TC and Paredes A: Plasma restoration of endothelial glycocalyx in a rodent model of hemorrhagic shock. *Anesthesia & Analgesia* 112(6):1289-95, 2011.

55. Kubulus D, Mathes A, Reus E, Pradarutti S, Pavlidis D, Thierbach JT, Heiser J, Wolf B, Bauer I and Rensing H: Endothelin-1 contributes to hemoglobin glutamer-200-mediated hepatocellular dysfunction after hemorrhagic shock. *Shock* 32(2):179-89, 2009.

56. Kumar A, Sen AP, Saxena PR and Gulati A: Resuscitation with diaspirin crosslinked hemoglobin increases cerebral and renal blood perfusion in hemorrhaged rats. *Artificial Cells, Blood Substitutes, & Immobilization Biotechnology* 25(1-2):85-94, 1997.

57. Maier M, Wackerle M, Herzog C and Marzi I: Supplementary administration of serum protein solution during shock resuscitation in the rat. *European Journal of Trauma* 30(5):289-295, 2004.
58. Maier S, Holz-Holzl C, Pajk W, Ulmer H, Hengl C, Dunser M, Haas T, Velik-Salchner C, Fries D, Greiner A, Hasibeder W and Knotzer H: Microcirculatory parameters after isotonic and hypertonic colloidal fluid resuscitation in acute hemorrhagic shock. *Journal of Trauma-Injury Infection & Critical Care* 66(2):337-45, 2009.
59. Mazzoni MC, Borgstrom P, Intaglietta M and Arfors KE: Capillary narrowing in hemorrhagic shock is rectified by hyperosmotic saline-dextran reinfusion. *Circulatory Shock* 31(4):407-18, 1990.
60. Messmer C, Yalcin O, Palmer AF and Cabrales P: Small-volume resuscitation from hemorrhagic shock with polymerized human serum albumin. *American Journal of Emergency Medicine* 30(8):1336-46, 2012.
61. Ni QY, Huang YX, Xu JY and Qiu HB: Effects of different fluid resuscitations on mesenteric microcirculation in rabbits of acute hemorrhagic shock. [Chinese]. *National Medical Journal of China* 93(9):693-697, 2013.
62. Nolte D, Botzlar A, Pickelmann S, Bouskela E and Messmer K: Effects of diaspirin-cross-linked hemoglobin (DCLHb) on the microcirculation of striated skin muscle in the hamster: a study on safety and toxicity. *Journal of Laboratory & Clinical Medicine* 130(3):314-27, 1997.
63. Ortiz D, Barros M, Yan S and Cabrales P: Resuscitation from hemorrhagic shock using polymerized hemoglobin compared to blood. *American Journal of Emergency Medicine* 32(3):248-55, 2014.

64. Paes-da-Silva F, Gonzalez AP and Tibirica E: Effects of fluid resuscitation on mesenteric microvascular blood flow and lymphatic activity after severe hemorrhagic shock in rats. *Shock* 19(1):55-60, 2003.
65. Palmer AF, Zhang N, Zhou Y, Harris DR and Cabrales P: Small-volume resuscitation from hemorrhagic shock using high-molecular-weight tense-state polymerized hemoglobins. *Journal of Trauma-Injury Infection & Critical Care* 71(4):798-807, 2011.
66. Pascual JL, Ferri LE, Chaudhury P, Seely AJE, Campisi G, Giannias B, Evans DC and Christou NV: Hemorrhagic shock resuscitation with a low molecular weight starch reduces neutrophil-endothelial interactions and vessel leakage in vivo. *Surgical Infections* 2(4):275-288, 2001.
67. Pascual JL, Ferri LE, Seely AJ, Campisi G, Chaudhury P, Giannias B, Evans DC, Razek T, Michel RP and Christou NV: Hypertonic saline resuscitation of hemorrhagic shock diminishes neutrophil rolling and adherence to endothelium and reduces in vivo vascular leakage.[Erratum appears in Ann Surg. 2003 Jan;237(1):148]. *Annals of Surgery* 236(5):634-42, 2002.
68. Paxian M, Keller SA, Huynh TT and Clemens MG: Perflubron emulsion improves hepatic microvascular integrity and mitochondrial redox state after hemorrhagic shock. *Shock* 20(5):449-57, 2003.
69. Peruski AM, Cooper ES and Butler AL: Microcirculatory effects of a hyperviscous hemoglobin-based solution administered intravenously in dogs with experimentally induced hemorrhagic shock. *American Journal of Veterinary Research* 75(1):77-84, 2014.

70. Sakai H, Hara H, Tsai AG, Tsuchida E, Johnson PC and Intaglietta M: Changes in resistance vessels during hemorrhagic shock and resuscitation in conscious hamster model. *Am J Physiol* 276(2 Pt 2):H563-71, 1999.
71. Sakai H, Takeoka S, Wettstein R, Tsai AG, Intaglietta M and Tsuchida E: Systemic and microvascular responses to hemorrhagic shock and resuscitation with Hb vesicles. *American Journal of Physiology - Heart & Circulatory Physiology* 283(3):H1191-9, 2002.
72. Scalia S, Burton H, Van Wylen D, Steinberg S, Hoffman A, Roche F and Flint L: Persistent arteriolar constriction in microcirculation of the terminal ileum following moderate hemorrhagic hypovolemia and volume restoration. *Journal of Trauma-Injury Infection & Critical Care* 30(6):713-8, 1990.
73. Torres L, Salgado C, Valdez C, Sondeen J, Dubick M and Filho IT: Protective function of endothelial glycocalyx (EG) during hemorrhagic shock (HS) in skeletal muscle: Integration of systemic and local parameters in vivo. *FASEB Journal* 29, 2015.
74. Torres LN, Salgado C, Valdez C, Sondeen JL, Dubick MA and Torres Filho I: Optimizing combat casualty care: Modulation of microvascular endothelium by resuscitation fluids after severe blood loss in rats. *Shock* 1):74-75, 2015.
75. Torres LN, Sondeen JL, Dubick MA and Filho IT: Systemic and microvascular effects of resuscitation with blood products after severe hemorrhage in rats. *Journal of Trauma and Acute Care Surgery* 77(5):716-723, 2013.
76. Torres LN, Sondeen JL, Ji L, Dubick MA and Torres Filho I: Evaluation of resuscitation fluids on endothelial glycocalyx, venular blood flow, and coagulation function after hemorrhagic shock in rats. *The Journal of Trauma and Acute Care Surgery* 75(5):759-66, 2013.

77. Vajda K, Szabo A and Boros M: Heterogeneous microcirculation in the rat small intestine during hemorrhagic shock: quantification of the effects of hypertonic-hyperoncotic resuscitation. *European Surgical Research* 36(6):338-44, 2004.
78. Vazquez BYS, Hightower CM, Martini J, Messmer C, Friesenecker B, Corbels P, Tsai AG and Intaglietta M: Vasoactive hemoglobin solution improves survival in hemodilution followed by hemorrhagic shock. *Critical Care Medicine* 39(6):1461-1466, 2011.
79. Villela NR, Cabrales P, Tsai AG and Intaglietta M: Microcirculatory effects of changing blood hemoglobin oxygen affinity during hemorrhagic shock resuscitation in an experimental model. *Shock* 31(6):645-52, 2009.
80. Villela NR, Tsai AG, Cabrales P and Intaglietta M: Improved resuscitation from hemorrhagic shock with Ringer's lactate with increased viscosity in the hamster window chamber model. *Journal of Trauma - Injury, Infection and Critical Care* 71(2):418-424, 2011.
81. Vollmar B, Lang G, Menger MD and Messmer K: Hypertonic hydroxyethyl starch restores hepatic microvascular perfusion in hemorrhagic shock. *American Journal of Physiology* 266(5 Pt 2):H1927-34, 1994.
82. Vollmar MD, Preissler G and Menger MD: Small-volume resuscitation restores hemorrhage-induced microcirculatory disorders in rat pancreas. *Critical Care Medicine* 24(3):445-50, 1996.
83. von Dobschuetz E, Hoffmann T and Messmer K: Diaspirin cross-linked hemoglobin effectively restores pancreatic microcirculatory failure in hemorrhagic shock. *Anesthesiology* 91(6):1754-62, 1999.

84. Wettstein R, Cabrales P, Erni D, Tsai AG, Winslow RM and Intaglietta M: Resuscitation from hemorrhagic shock with MalPEG-albumin: Comparison with MalPEG-hemoglobin. *Shock* 22(4):351-357, 2004.
85. Wettstein R, Erni D, Intaglietta M and Tsai AG: Rapid restoration of microcirculatory blood flow with hyperviscous and hyperoncotic solutions lowers the transfusion trigger in resuscitation from hemorrhagic shock. *Shock* 25(6):641-6, 2006.
86. Wettstein R, Tsai AG, Erni D, Lukyanov AN, Torchilin VP and Intaglietta M: Improving microcirculation is more effective than substitution of red blood cells to correct metabolic disorder in experimental hemorrhagic shock. *Shock* 21(3):235-40, 2004.
87. Wettstein R, Tsai AG, Erni D, Winslow RM and Intaglietta M: Resuscitation with polyethylene glycol-modified human hemoglobin improves microcirculatory blood flow and tissue oxygenation after hemorrhagic shock in awake hamsters. *Critical Care Medicine* 31(6):1824-30, 2003.
88. Wu C-Y, Chan K-C, Cheng Y-J, Yeh Y-C and Chien C-T: Effects of different types of fluid resuscitation for hemorrhagic shock on splanchnic organ microcirculation and renal reactive oxygen species formation. *Critical Care* 19(1):1-13, 2015.
89. Wu CY, Yeh YC, Chien CT, Chao A, Sun WZ and Cheng YJ: Laser speckle contrast imaging for assessing microcirculatory changes in multiple splanchnic organs and the gracilis muscle during hemorrhagic shock and fluid resuscitation. *Microvasc Res* 101:55-61, 2015.
90. Yada-Langui MM, Anjos-Valotta EA, Sannomiya P, Rocha ESM and Coimbra R: Resuscitation affects microcirculatory polymorphonuclear leukocyte behavior after

hemorrhagic shock: Role of hypertonic saline and pentoxifylline. *Experimental Biology and Medicine* 229(7):684-693, 2004.

91. Zakaria ER, Tsakadze NL and Garrison RN: Hypertonic saline resuscitation improves intestinal microcirculation in a rat model of hemorrhagic shock. *Surgery* 140(4):579-588, 2006.

92. Zhao L, Wang B, You G, Wang Z and Zhou H: Effects of different resuscitation fluids on the rheologic behavior of red blood cells, blood viscosity and plasma viscosity in experimental hemorrhagic shock. Vol. 80. 2009, pp. 253-258.

93. De Backer D, Donadello K and Cortes DO: Monitoring the microcirculation. *J Clin Monit Comput* 26(5):361-6, 2012.

94. Wang P, Hauptman JG and Chaudry IH: Hemorrhage produces depression in microvascular blood flow which persists despite fluid resuscitation. *Circulatory Shock* 32(4):307-18, 1990.

95. Hutchings S, Wendon J, Watts S and Kirkman E: Microcirculatory and macrocirculatory responses in a porcine model of traumatic haemorrhagic shock and resuscitation. *British Journal of Anaesthesia* 112 (1):185P-186P, 2014.

96. Wan Z, Sun S, Ristagno G, Weil MH and Tang W: The cerebral microcirculation is protected during experimental hemorrhagic shock. *Critical Care Medicine* 38(3):928-32, 2010.

97. Lu H, Zheng J, Zhao P, Zhang G and Wu T: Buccal partial pressure of carbon dioxide outweighs traditional vital signs in predicting the severity of hemorrhagic shock in a rat model. *Journal of Surgical Research* 187(1):262-9, 2014.

98. Hightower CM, Salazar Vazquez BY, Cabrales P, Tsai AG, Acharya SA and Intaglietta M: Plasma expander and blood storage effects on capillary perfusion in transfusion after hemorrhage. *Transfusion* 53(1):49-59, 2013.

99. Machiedo GW, Zaets SB, Berezina TL, Xu DZ, Feketova E, Spolarics Z and Deitch EA: Trauma-hemorrhagic shock-induced red blood cell damage leads to decreased microcirculatory blood flow. *Critical Care Medicine* 37(3):1000-10, 2009.
100. Sakai H, Tsai AG, Kerger H, Park SI, Takeoka S, Nishide H, Tsuchida E and Intaglietta M: Subcutaneous microvascular responses to hemodilution with a red cell substitute consisting of polyethyleneglycol-modified vesicles encapsulating hemoglobin. *J Biomed Mater Res* 40(1):66-78, 1998.
101. Cabrales P, Tsai AG and Intaglietta M: Microvascular pressure and functional capillary density in extreme hemodilution with low- and high-viscosity dextran and a low-viscosity Hb-based O₂ carrier. *American Journal of Physiology - Heart and Circulatory Physiology* 287(1 56-1):H363-H373, 2004.
102. Varga R, Torok L, Szabo A, Kovacs F, Keresztes M, Varga G, Kaszaki J and Boros M: Effects of colloid solutions on ischemia-reperfusion-induced periosteal microcirculatory and inflammatory reactions: Comparison of dextran, gelatin, and hydroxyethyl starch. *Critical Care Medicine* 36(10):2828-2837, 2008.
103. Simonian GT, Dardik H, Hallac D, Mazziotta R, Ibrahim I and Stahl R: Hemodynamic and histopathologic effects of hydroxyethyl starch and superoxide dismutase following splanchnic arterial occlusion in a murine model. *Vascular Surgery* 31(5):645-656, 1997.
104. Jonas J, Heimann A, Strecker U and Kempinski O: Hypertonic/hyperoncotic resuscitation after intestinal superior mesenteric artery occlusion: early effects on circulation and intestinal reperfusion. *Shock (Augusta, Ga.)* 14(1):24-29, 2000.
105. Nolte D, Bayer M, Lehr HA, Becker M, Krombach F, Kreimeier U and Messmer K: Attenuation of postischemic microvascular disturbances in striated

muscle by hyperosmolar saline dextran. *American Journal of Physiology* 263(5 Pt 2):H1411-6, 1992.

106. Arslan E, Sierko E, Waters JH and Siemionow M: Microcirculatory hemodynamics after acute blood loss followed by fresh and banked blood transfusion. *American Journal of Surgery* 190(3):456-462, 2005.

107. Gonzalez AM, Yazici I, Kusza K and Siemionow M: Effects of fresh versus banked blood transfusions on microcirculatory hemodynamics and tissue oxygenation in the rat cremaster model. *Surgery* 141(5):630-639, 2007.

108. Kameneva MV, Wu ZJ, Uraysh A, Repko B, Litwak KN, Billiar TR, Fink MP, Simmons RL, Griffith BP and Borovetz HS: Blood soluble drag-reducing polymers prevent lethality from hemorrhagic shock in acute animal experiments. *Biorheology* 41(1):53-64, 2004.

109. Marzi I, Maier M, Herzog C and Bauer M: Influence of pentoxifylline and albifylline on liver microcirculation and leukocyte adhesion after hemorrhagic shock in the rat. *Journal of Trauma-Injury Infection & Critical Care* 40(1):90-6, 1996.

110. Singh G, Chaudry KI and Chaudry IH: Diltiazem reduces whole blood viscosity following trauma-hemorrhagic shock and resuscitation. *Circulatory Shock* 39(3):231-6, 1993.

111. Shtykhno lu M, Khugaeva VK and Donskikh EA: [Therapeutic effectiveness and the effect on the microcirculation of different blood substitutes in the terminal phase of experimental traumatic shock]. *Probl Gematol Pereliv Krovi* 23(6):26-30, 1978.

112. Lima R, Villela NR and Bouskela E: Microcirculatory effects of selective receptor blockade during hemorrhagic shock treatment with vasopressin: experimental study in the hamster dorsal chamber. *Shock* 38(5):493-8, 2012.

113. Sheng C, Yu YH, Zhao KS, Qin W and Wang CH: Hypotensive resuscitation combined with polydatin improve microcirculation and survival in a rabbit model of uncontrolled hemorrhagic shock in pregnancy. *Journal of Surgical Research* 168(1):103-10, 2011.
114. Bauer C, Kuntz W, Ohnsmann F, Gasser H, Weber C, Redl H and Marzi I: The attenuation of hepatic microcirculatory alterations by exogenous substitution of nitric oxide by s-nitroso-human albumin after hemorrhagic shock in the rat. *Shock (Augusta, Ga.)* 21(2):165-169, 2004.
115. Zhao KS, Zhu ZG, Woo GY and Haun XL: Effect of naloxone on microcirculatory behavior during irreversible hemorrhagic shock. *Microvascular Research* 34(1):84-95, 1987.
116. Pati S, Matijevic N, Doursout MF, Ko T, Cao Y, Deng X, Kozar RA, Hartwell E, Conyers J and Holcomb JB: Protective effects of fresh frozen plasma on vascular endothelial permeability, coagulation, and resuscitation after hemorrhagic shock are time dependent and diminish between days 0 and 5 after thaw. *Journal of Trauma-Injury Infection & Critical Care* 69 Suppl 1:S55-63, 2010.
117. Savage SA, Fitzpatrick CM, Kashyap VS, Clouse WD and Kerby JD: Endothelial dysfunction after lactated Ringer's solution resuscitation for hemorrhagic shock. *Journal of Trauma-Injury Infection & Critical Care* 59(2):284-90, 2005.
118. Fitzpatrick CM, Savage SA, Kerby JD, Clouse WD and Kashyap VS: Resuscitation with a blood substitute causes vasoconstriction without nitric oxide scavenging in a model of arterial hemorrhage. *Journal of the American College of Surgeons* 199(5):693-701, 2004.

119. Van Meurs M, Wulfert FM, Knol AJ, De Haes A, Houwertjes M, Aarts LPHJ and Molema G: Early organ-specific endothelial activation during hemorrhagic shock and resuscitation. *Shock* 29(2):291-299, 2008.
120. Makley AT, Goodman MD, Friend LAW, Deters JS, Johannigman JA, Dorlac WC, Lentsch AB and Pritts TA: Resuscitation with fresh whole blood ameliorates the inflammatory response after hemorrhagic shock. *Journal of Trauma - Injury, Infection and Critical Care* 68(2):305-310, 2010.
121. Holcomb JB, Tilley BC, Baraniuk S, Fox EE, Wade CE, Podbielski JM, del Junco DJ, Brasel KJ, Bulger EM, Callcut RA, Cohen MJ, Cotton BA, Fabian TC, Inaba K, Kerby JD, Muskat P, O'Keeffe T, Rizoli S, Robinson BR, Scalea TM, Schreiber MA, Stein DM, Weinberg JA, Callum JL, Hess JR, Matijevic N, Miller CN, Pittet JF, Hoyt DB, Pearson GD, Leroux B and van Belle G: Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. *Jama* 313(5):471-82, 2015.
122. Cooper ES, Bateman SW and Muir WW: Evaluation of hyperviscous fluid resuscitation in a canine model of hemorrhagic shock: a randomized, controlled study. *J Trauma* 66(5):1365-73, 2009.
123. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L and Sterne JA: The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *Bmj* 343:d5928, 2011.
124. Dwan K, Gamble C, Williamson PR, Kirkham JJ and Reporting Bias G: Systematic review of the empirical evidence of study publication bias and outcome reporting bias - an updated review. *PLoS One* 8(7):e66844, 2013.